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A NEW EYE COLOR MUTANT IN THE MOUSE WITH ASYMMETRICAL EXPRESSION

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Most hereditary variations in the paired structures of animals with bilateral symmetry express themselves equally on both sides of the body. Exceptions to this rule are of interest as potential material for revealing new relationships in the mechanism of development. One such exception has been found recently in which an eye color mutation in the mouse frequently expresses itself differently in the right and left eyes.

The observations began while we were studying a case of *heterochromia iridis* which had been noted in a single animal in our stocks. This animal, a brown agouti pied female with a normal brown left eye and a much lighter rose- or ruby-colored right eye proved to be a somatic mosaic with no demonstrable change in the germ cells since we found no eye color variations in 6 F_1 and 31 F_2 offspring from this female. In searching for other possible occurrences of this type of variation we found that Dr. C. H. Danforth of Stanford University was maintaining a stock of silver mice with *heterochromia iridis*, a frequent condition being one eye ruby, the other pink. Dr. Danforth kindly sent us several animals from this stock which we tested by crossing with our heterochromic female. The F_1 consisted of normal black-eyed animals only, indicating that the two occurrences of heterochromia were not due to the same germinal change. In F_2, some animals with ruby eyes like those in the Danforth parent stock appeared, indicating segregation of a recessive ruby mutation.

We then turned our attention to the ruby mutation which had not been described previously. It proved to be a simple recessive to normal, outcrosses to wild type producing only wild type eye color in F_1 and 179 wild type (dark-eyed) and 54 ruby-eyed in F_2. The new eye color was easily scored at birth, the eyes of newborn mutants lacking the iris pigment ring of the wild type and resembling the unpigmented eyes of the mutants pink, pallid and albino.

The F_2 mutants obtained above contained the ruby gene (r) in combination with agouti and with black. Black agouti-ruby superficially resembles brown agouti since the black base of the fur is reduced to dark sepia while the yellow tip is only slightly reduced in intensity. Black-ruby is dull dark sepia or dark slate. Ruby obviously affects black hair pigments in the same direction but to a lesser extent than the mutants pink and pallid, while yellow which is unaffected by the latter mutants is somewhat reduced by ruby. Of the F_2 ruby animals nearly all showed ruby on both sides, but a few showed the heterochromia of the ruby grandparent.

These effects all resemble those of a mutation reported by Sô and Imai.[1] According to Grüneberg[2] (p. 42), who has had the Japanese text of this paper translated, the evidence shows that the ruby mutation of Sô and Imai was an allele of pink, the order of dominance being $P \gg p^r > p$. I have also examined a translation of this paper* and agree with Grüneberg that the evidence for allelism is conclusive. Consequently our ruby was tested by a pink-eyed agouti stock. F_1 was normal dark-eyed; F_2 consisted of 56 normal, 24 ruby-eyed and 28 pink-eyed. This is a sufficiently close approach to 9:3:4 ratio to suggest that our ruby and pink assort independently, the double recessive *pp rr* being pink-eyed. A number of F_2 pink-eyed animals were tested. While most of them proved to be *pp Rr*, a few were *pp rr*. A double recessive stock of pink-eyed animals *pp rr* was prepared of which the members were indistinguishable in eye color or coat color from the normal pink-eyed stock. Linkage relations of the new ruby with pink were tested by matings $\frac{PR}{pr} \times pp\ rr$ and $\frac{Pr}{pR} \times pp\ rr$. The former produced 36 dark-eyed (*PR*) and 87 light-eyed (*pR*, *Pr* and *pr*) young; the latter, 35 dark-eyed and 91 light-eyed. Combining these, the recombinations, 128, constituted almost exactly half of the 249 gametes tested, indicating free assortment of p and r.

Since two phenotypically indistinguishable but independent pink-eye mutations have been recorded in the mouse, it occurred to us that the Japanese investigators may have employed the p^2 (pallid) allele in their tests. We therefore tested ruby by p^2.† This produced only wild type offspring (38 observed) indicating that r is not a mutation at the p^2 locus. Similar tests by albino likewise gave no evidence of allelism of r and c^a. We may therefore assume that r marks a new locus in the mouse.

For reasons of interest in this laboratory we tested r for location in the T (Brachyury) chromosome. The tests produced the following results: $\frac{+T}{r+} \times ++$ gave 100 ++, 114 + T, 124 $r+$, 100 r T. This fits an assumption of independent assortment ($p = 0.3$) and indicates that the r locus is probably not in the T chromosome.

Returning now to the asymmetrical expression of the new mutant we have noted that in the original stock as received from Professor Danforth, 53 animals carefully examined showed the following eye color distribution, as compared with rubies extracted from outcrosses to non-ruby stocks:

	BOTH EYES RUBY	LEFT EYE RUBY RIGHT EYE PINK	LEFT EYE PINK RIGHT EYE RUBY	BOTH EYES PINK
Original stock	22	11 + 5[a]	5 + 5[b]	5
Extracted ruby	50	4	3	1

[a] These were recorded as left eye dark ruby, right eye light ruby.
[b] These were recorded as right eye dark ruby, left eye light ruby.

While it is clear from these observations that asymmetry in eye color is frequent in the original stock (26 asymmetrical out of 53 carefully observed for this character), it is not evident that the asymmetry is due to the ruby gene, since after extraction from outcrosses the same ruby gene showed only about 12 per cent (7/57) asymmetry. The original stock was silver black pied (*aa BB sisi ss*). After outcrossing this to black agouti (*AA BB SiSi SS*), black and tan (a^ta^t *BB SiSi SS*) and pink-eyed black stocks (*aa BB pp SiSi SS*) the asymmetry of extracted ruby-eyed animals showed no relation to *A*, a^t or *a*, nor to the *Si-si* alleles. However out of 7 asymmetrical rubies, 5 were pied. Since the total number of pied animals observed in segregating generations was 9, we have proportions of asymmetrical ruby-eyed as 5/9 among pied and 2/48 among non-pied. The difference between these proportions is highly significant ($p = < 0.001$). It is probable therefore that the asymmetrical expression of ruby is greatly enhanced by pied spotting. This is to be taken as a preliminary indication only, since extended and systematic studies of other factors influencing asymmetrical expression have not been made.

In the present data there is some tendency for the left eye to be darker than the right, but the observations are too few to provide a test of this point. It is apparent that the expression of the ruby mutation depends upon an equilibrium which is influenced by both genetic and developmental factors which deserve further study.

The ruby mutation of Sô and Imai was similarly quite variable in density of eye color and this appeared to depend on other (unanalyzed) genetic factors. The Japanese authors also noted *heterochromia iridis* frequently in the ruby-eyed stock. They believed it to be genetically influenced but because of the weakness of such animals were unable to analyze it.

The mutation of Sô and Imai and that described here are the only two in mice in which heterochromia has been observed. This fact and their other similarities render them phenotypically almost identical yet they appear to be mutations at different loci. The only alternative to this is to assume

that the pink mutation with which Sô and Imai's ruby proved to be allelic was a third mutation, different from the p and p^2 of American and European stocks. This is unlikely since the original pink mutation was introduced into Europe from Japan.

* Made by Toshi Miyazaki of Columbia University.

† Kindly supplied by Jackson Memorial Laboratory.

[1] Sô, M., and Imai, Y., "On the Inheritance of Ruby Eye in Mice," *Japanese Jour. Genet.*, **4**, 8–9 (1926).

[2] Grüneberg, H., *The Genetics of the Mouse*, University Press, Cambridge, 1943, 412 pages.

ON NON-LINEAR PHENOMENON OF SELF-ROLLING

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Recently developed methods of non-linear mechanics offer interesting applications to a number of problems which have been known for a long time but which have remained unexplored so far. A typical problem of this kind is the so-called *self-rolling* of a ship provided with an antirolling stabilizing system. It is well known that, if the phase of the control action is reversed, the ship, originally at rest in still water, begins to roll with gradually increasing roll angles until the amplitude reaches a stationary value after which the self-rolling continues indefinitely. Although the physical nature of this phenomenon is sufficiently clear, it is impossible to determine the stationary amplitude from the linear theory for what eventually limits the amplitude is precisely the action of the various non-linearities which are generally left out in a simplified linear theory.

The differential equation of rolling in still water is

$$I\ddot{\theta} + a_1'\dot{\theta} + a_2'\dot{\theta}^2 + Dh(\theta) = 0 \qquad (1)$$

where θ is the angle of roll, D is the displacement of the ship, I is the virtual moment of inertia about the longitudinal axis through the center of gravity, a_1' and a_2' are the so-called Froude's coefficients of resistance to rolling (bilge keels, skin friction), and $h(\theta)$ is the lever arm of the restoring couple. For small angles of roll $h(\theta) \cong h_1\,\theta$ where h_1 is the initial metacentric height assumed to be constant. For larger angles one can approximate the quantity $Dh(\theta)$ by an expression of the form

$$Dh(\theta) = c_1'\theta - c_3'\theta^3 \qquad (2)$$

which means that the restoring couple decreases for large θ. The coefficients c_1' and c_3' depend on the form of the hull and can be computed for a given ship.

The stabilizing moment S in general is a certain function of $\dot{\theta}$. In practice one endeavors to obtain a linear law $S = b\dot{\theta}$, where b is a constant. In reality owing to the usual form of the characteristic of the stabilizing power plant it is found[1] that the stabilizing moment for larger $\dot{\theta}$ increases less rapidly than according to the above simple relation and can, therefore, be approximated also by an expression of the form

$$S = b_1'\dot{\theta} - b_3'\dot{\theta}^3. \tag{3}$$

The coefficients b_1' and b_4' can be determined from the characteristic of the equipment. Since we are interested here in self-rolling, we have to add $-S$ on the left side of equation (1). The differential equation of self-rolling will then be

$$I\ddot{\theta} + a_1'\dot{\theta} + a_2'\dot{\theta}^2 - b_1'\dot{\theta} + b_3'\dot{\theta}^3 + c_1'\theta - c_3'\theta^3 = 0. \tag{4}$$

Dividing by I and setting $c_1'/I = \omega_1^2$; $a_1'/I = a_1$; $a_2'/I = a_2$, etc., this equation becomes

$$\ddot{\theta} + \omega_1^2\theta = (b_1 - a_1)\dot{\theta} - a_2\dot{\theta}^2 - b_3\dot{\theta}^3 + c_3\theta^3 = f(\theta, \dot{\theta}). \tag{5}$$

This is the non-linear differential equation of our problem. We will assume that the upper bound of the non-linear function $f(\theta, \dot{\theta})$ is small so that the non-linear motion specified by equation (5) remains in the neighborhood of the linear motion corresponding to $f(\theta, \dot{\theta}) = 0$. This assumption is justified in practice since it is observed that the self-excited stationary oscillation is practically sinusoidal. Owing to this assumption it is possible to apply the so-called method of the first approximation[2] and solve the problem.

We shall leave out the case when $b_1 - a_1 = \alpha < 0$ as no self-rolling is possible in this case and will consider only the case when $\alpha > 0$, that is, when the linear term $b_1\dot{\theta}$ due to the stabilizer outweighs the linear term $a_1\dot{\theta}$ of the Froude resistance to rolling. One ascertains this from the theory of Poincaré,[3] viz.: $\alpha = 0$ is a bifurcation point; for $\alpha < 0$ the singular point of equation (5) is a stable focal point; for $\alpha > 0$ it is an unstable focal point which means that the amplitudes begin to increase from rest.

In order to obtain the stationary amplitude $\bar{\theta}_0$ we look for a solution of the form $\theta = \theta_0 \sin \psi$, where θ_0 is an amplitude (not necessarily stationary) and $\psi = \omega_0 t + \varphi$ is the so-called *total phase*. The quantities θ_0 and ψ are determined by the differential equations

$$\frac{d\theta_0}{dt} = \frac{1}{2\pi\omega_0}\int_0^{2\pi} f(\theta_0 \sin\lambda, \theta_0\omega_0 \cos\lambda)\cos\lambda d\lambda = \frac{1}{2\pi\omega_0}\phi(\theta_0) \tag{6}$$

$$\frac{d\psi}{dt} = \Omega(\theta_0) = \omega_0 - \frac{1}{2\pi\theta_0\omega_0}\int_0^{2\pi} f(\theta_0 \sin\lambda, \theta_0\omega_0 \cos\lambda) \sin\lambda d\lambda$$

$$= \omega_0 - \frac{1}{2\pi\theta_0\omega_0}\Psi(\theta_0). \quad (7)$$

The condition for a stationary amplitude is

$$\phi(\bar{\theta}_0) = 0. \quad (8)$$

In carrying out these calculations attention must be paid to the term $a_2\dot{\theta}^2$ in equation (5). In the integration between 0 and 2π, this term, for obvious physical reasons must be written as $a_2|\dot{\theta}|\dot{\theta}$. When all integrations are made, condition (8), upon the cancellation of the unstable solution $\bar{\theta}_0 = 0$ reduces to the following

$$^3/_4 b_3\pi\omega_0{}^3\bar{\theta}_0{}^2 + {}^8/_3 a_2\omega_0{}^2\bar{\theta}_0 - \alpha\omega_0\pi = 0. \quad (9)$$

The stationary amplitude $\bar{\theta}_0$ is given by the positive root of this equation, viz.:

$$\bar{\theta}_0 = \frac{16}{9\pi\omega_0}\frac{a_2}{b_3}\left[\sqrt{1 + \frac{27}{64}\pi^2\frac{\alpha b_3}{a_2{}^2}} - 1\right]. \quad (10)$$

From this expression it follows that for $\alpha < 0$ there exists no positive value for $\bar{\theta}_0$ which means that the self excitation is impossible. This follows also from the theory of Poincaré as was just mentioned. For very small positive value of α we have

$$\bar{\theta}_0 \cong \frac{3}{8}\frac{\pi\alpha}{\omega_0 a_2}. \quad (11)$$

Hence, for a relatively small intensity of the self-rolling control, the stationary amplitude increases in proportion to α. If α remains fixed in that range, the stationary amplitude is in an inverse proportion to the coefficient a_2. The frequency correction ω_1 is obtained from equation (7). Carrying out the indicated integrations one obtains

$$\Omega(\bar{\theta}_0) = \omega_0 - \omega_1 = \omega_0 - \frac{3c_3}{8\omega_0}\bar{\theta}_0{}^2 \quad (12)$$

where $\bar{\theta}_0$ is given by equations (10) or (11). It is seen thus that the non-linear stationary frequency is somewhat lower than the corresponding linear undamped frequency ω_0. The frequency correction ω_1 depends on $\bar{\theta}_0$.

If the non-linearities (a_2 for the ship and b_3 for the stabilizer) are known, equation (10) permits determining $\bar{\theta}_0$. If, however, a_2 is unknown and the stabilizing equipment is available, by observing $\bar{\theta}_0$ and computing b_3 it is possible to determine the Froude constant a_2.

[1] Minorsky, N., "On Mechanical Self-Excited Oscillations," *Proc. Nat. Acad. Sci.*, **30**, 308–314 (1944).

[2] Kryloff, N., and Bogoliuboff, N., "Introduction à la Mécanique non linéaire," Kieff (1937); also free English translation of S. Lefschetz, Princeton University Press (1943).

[3] Poincaré, H., *"Oeuvres,"* Paris, Volume 1; also D. Taylor Model Basin report No. 534 (1944).

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*EFFECTS OF EXPOSURE TO ULTRA-VIOLET LIGHT ON SUBSEQUENT DARK ADAPTATION**

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It has been shown that ultra-violet light below 365 mμ affects the visual mechanism in baby chicks by raising the threshold for recognition of flicker. For the return of the threshold to its normal level many hours are required.[1]

For the human eye it is known that the course of dark adaptation can be modified by previous exposure to different levels of intensity[2] and to selected portions of the visible spectrum of the same photic intensity.[3] The influence of the ultra-violet component of light has thus far not been taken into consideration. With data on effects of ultra-violet on the chick's eye available, its dark adaptation was studied after previous exposure to lights of varying ultra-violet content.

Preceding dark adaptation measurements, chicks 4 to 12 days old are exposed singly to three 250 watt mercury vapor lamps (GE type H-5), while confined to a cylindrical wire mesh cage, but free to move with access to food and water. The lamps are mounted at eye level, equally spaced outside the cage and surrounded by a reflecting surface. The illumination at the center of the cage is 880 millilamberts as measured with a Weston photoelectric photometer. Lamp housings permit the insertion of filters for selection of specific spectral regions. For exposure to the light a standard period of 10 minutes is chosen.

With the completion of light exposure the chick is transferred rapidly into a cylindrical glass cage and brought into the apparatus for testing responses to flicker.[4] Flicker is produced by a cylindrical system of equally spaced dark vertical bars, rotating around the animal. At threshold level of light intensity the chick responds with a movement of its head, following the direction of the stripe motion through an angle of 45 to 90° and returning abruptly to its initial position (head nystagmus). In determining the level of dark adaptation at any given moment it is important that the width of the bars be big enough to be seen at minimum intensities.

In our case the visual angle of each bar measures 36° at the center of the glass cage. Flicker recognition also depends upon the velocity of passage of the bars in front of the eye and must be slow at threshold intensities. The speed chosen is therefore the slowest possible at which precise responses occur, and this is about 1 to 1.5 flicker per second. The first test is made one minute after cessation of illumination and the threshold intensity for response recorded. Then the chick remains in complete darkness and the tests are repeated every minute up to 10, then every two

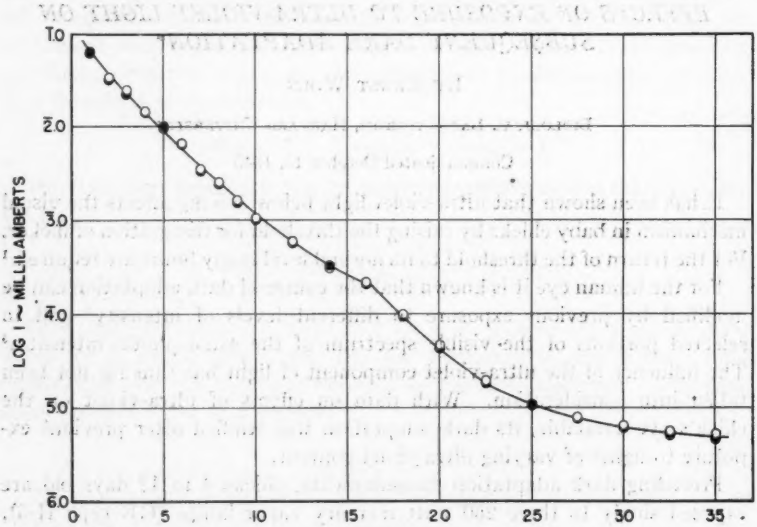

FIGURE 1

Relations of threshold intensities of illumination and time of dark adaptation for the eye of the baby chick, after exposure to white light (880 millilamberts) for 10 minutes. Two series of measurements, each containing six individuals, are represented by different symbols.

minutes up to 20, and every 2.5 minutes up to 35 minutes of dark adaptation. For each dark adaptation curve plotted the averaged readings of six individuals are taken to smooth out individual deviations.

Detrimental effects of ultra-violet light on visual threshold, as described previously,[1] showed that wave-lengths below 365 mμ raise the threshold, whereas longer waves have no effect. For a normal dark adaptation curve it is therefore necessary to filter out from the exposure light any portion of the ultra-violet that might have an influence upon its course. A filter with the designation XY 91 VO of the Polaroid Corporation which trans-

mits only wave-lengths above 400 mμ and does not produce any noticeable changes of hue in the visible was selected to provide the exposure light for our standard curve.

In figure 1 the mean values of threshold intensities for response are represented. The curve has the general form of a dark adaptation curve typical for a visually duplex vertebrate eye (cf. human eye). In darkness the sensitivity increases rapidly in the beginning, then more slowly, until it reaches a relatively steady level after about 35 minutes, when the experiment is broken off. Due to the presence of rods and cones in the retina of the chick's eye, the curve shows a break dividing the process of adaptation into a cone and a rod component. The break, sharply indicated in the graph, because of the fact that the point at 16 minutes may equally well be fitted to either segment, would not be so sharp, if all individual readings were plotted; the transition would rather be rounded off. The fact that the break occurs after about 16 minutes of dark adaptation is of interest in so far as for the human eye (large visual field and white exposure light) the transition occurs much earlier.[5] There is also a considerable difference in relative extent of the cone and rod range. For the human eye, after light adaptation to intensities of the same order of magnitude, the cone range covers 1.5–2 log. units, the rod range 3 log. units. In the chick the cone range is 3 log. units, whereas the rod range is only 1.5. The relative participation of the two groups of receptors is roughly the reverse in the chick from that in man. These facts are in accordance with morphological differences between the human and the chick's eye. The retina of the chick, as in most diurnal birds, is predominantly a cone retina with the interspersion of relatively few rods, whereas the human retina has, besides the high concentration of cones in the fovea, relatively few cones distributed among the peripheral rods.[6]

Knowing the course and extent of dark adaptation of the chick's eye after exposure to ordinary light free of ultra-violet, we are in a position to add to the exposure light certain ranges of the ultra-violet spectrum by the use of different absorption filters. The range between 300 and 400 mμ is of particular interest. A series of filters with cut-offs at different wave-lengths in this region provided previously a differential influence[1] and is used here for a study of similar effects on the course of dark adaptation. The filters are in their order of transmission: Corning 774 (heat-resisting clear chemical glass), AO crown glass 1045, ordinary plate glass, AO Cruxite 1794, Corning 3850 (greenish Nultra), AO Calobar C-1827, AO 2614, Corning 3389 (Noviol shade A), XY 91 VO of the Polaroid Corporation and Corning 9863 (red-purple Corex A). The transmissions of these filters, determined with a Beckman spectrophotometer, are given in figure 2.

With Corning 774 (jacket of the GE H-5 lamp as furnished by the manufacturer) in front of the mercury vapor arc during light exposure, it is

found that in subsequent dark adaptation tests for the first 16 minutes the course is identical with the standard curve given in figure 1. From the point of cone-rod transition on, where we would normally expect a considerable drop in threshold intensities, only a slow decline is apparent and we arrive very soon at a steady level so that after 35 minutes in the dark it is about 1.3 log. units higher than the normal. Due to the presence of the ultra-violet in the exposure light, the state of dark adaptation is therefore only one-twelfth of the normal at the termination of the tests.

A gradual reduction of the extent of the ultra-violet band from 290 to 360 mμ by crown, plate glass, Cruxite and Corning 3850 affects the course and final threshold of dark adaptation in correspondence to the elimination of the shorter wave-lengths. At 35 minutes the difference in threshold intensity from the normal is for crown about 1.1, for plate glass 0.6, for

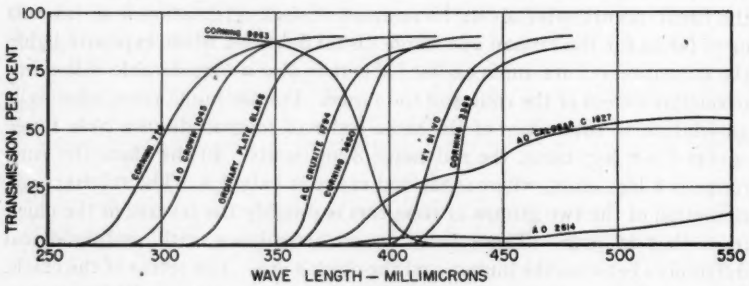

FIGURE 2

Spectrophotometric transmissions of filters used for addition of different ranges of ultra-violet to the white adapting light.

Cruxite 0.3 and for Corning 3850 0.2 log. units. All curves would, of course, after hours reach the same low level as our standard curve in figure 1, depending upon the velocity of recovery from the effect of the ultra-violet.

Exposure light which does not contain any wave-lengths shorter than 365 mμ results in dark adaptation curves which are identical with our standard curve. Corning 3389 has a cut-off at higher wave-lengths than XY 91 VO, and the experimental readings are found practically identical for both. AO Calobar which transmits about 1.5 per cent at 365 mμ and AO 2614 which begins to transmit at 380 mμ are both tinted glasses and have therefore a lower transmission in the visible compared with the colorless filters thus far considered. For Calobar the maximum transmission at 535 mμ is 55 per cent, for AO 2614 at 625 mμ about 20 per cent. Both reduce therefore the brightness of the exposure light. The effect of this decrease is noticeable when following the individual points along the curve;

they are consistently on the low side. The final thresholds for both filters fall closely within the scatter range of the normal curve. It may therefore

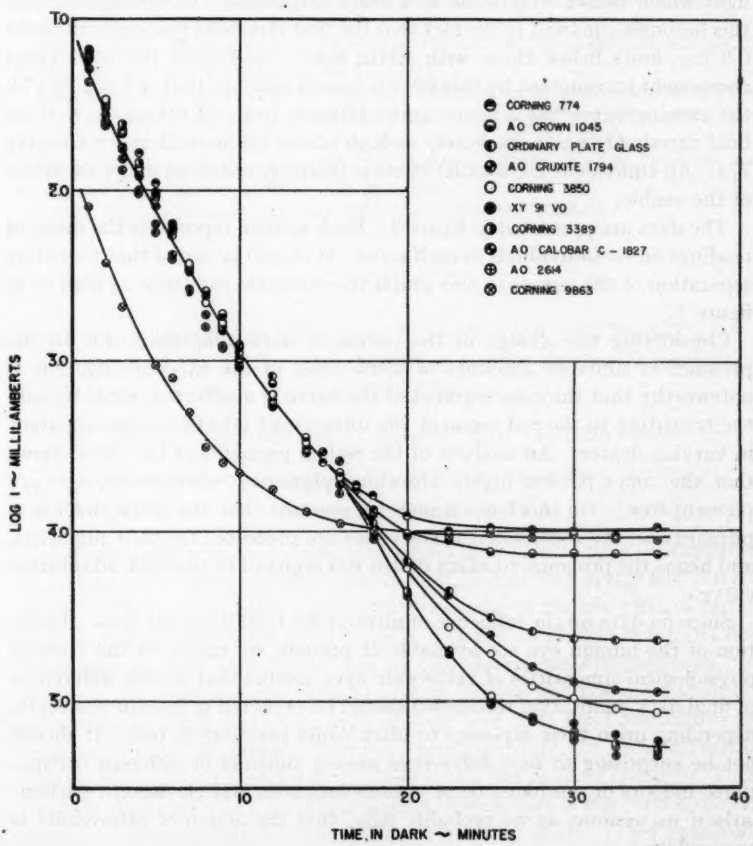

FIGURE 3

The course of dark adaptation of the eye of the baby chick, measured after preceding exposure to white light containing different ranges of the ultra-violet spectrum. In proportion to the increase of the ultra-violet component below 365 mμ the final dark adaptation thresholds are raised to higher intensities, indicating a reduction in potential dark adaptation by the ultra-violet. Light containing wave-lengths above 365 mμ does not produce such effects.

be assumed that any ultra-violet above 365 mμ has no effect upon the course of dark adaptation.

Of particular interest are the results with Corning filter 9863 in front of the exposure light. This filter transmits only a small portion of the far red,

a small amount of violet, but almost as much of the effective ultra-violet as Corning 774. During exposure to light the chick receives a dim purple light which causes adaptation to a lower brightness. In subsequent tests this becomes apparent in the fact that the first threshold readings fall about 0.9 log. units below those with white light. And since the ultra-violet component transmitted by this filter is almost equal to that of Corning 774, the ensuing curve has a shape quite different from all others, so that its final threshold values are nearly as high above the normal as for Corning 774. An equivalent ultra-violet effect is therefore obtained under exclusion of the visible.

The data are presented in figure 3. Each symbol represents the mean of readings on six individuals in each series. It should be noted that for better separation of the curves in this graph the ordinates are twice as high as in figure 1.

Considering the change in the course of dark adaptation due to the presence of different amounts of ultra-violet in the exposure light, it is noteworthy that the cone segment of the curve is unaffected, while beyond the transition to the rod segment the ultra-violet effect becomes apparent in varying degree. An analysis of the retinal pigments of the chick shows that the cones possess highly absorbing pigments, whereas the rods are pigment-free.[7] On this basis it may be assumed that the ultra-violet acts primarily on the rods, whereas the cones are protected by their pigments, and hence the pronounced effect on the rod segment of the dark adaptation curve.

Since no data on the influence of ultra-violet light upon the dark adaptation of the human eye are available at present, we might on the basis of physiological similarities of vertebrate eyes assume that similar differences in final dark adaptation thresholds should be expected in human observers, depending upon their exposure to ultra-violet previous to test. It should not be surprising to find differences among subjects of different occupations, indoors or outdoors, or at various latitudes and elevations, particularly if we assume, as we probably may, that the action of ultra-violet is cummulative.

Summary.—The course of dark adaptation in baby chicks is studied after exposure to white adapting light containing different amounts of ultra-violet radiation between 280 and 400 mμ. Exposure light of wavelengths longer than 365 mμ results in uniform dark adaptation curves. Any addition of ultra-violet below 365 mμ prevents complete adaptation, raising the final threshold considerably above the normal level. The difference between complete and partial dark adaptation due to exposure to the short waves is proportional to the extent of the ultra-violet spectrum.

* This research was supported by a grant of the American Optical Company.

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CARBON DIOXIDE UTILIZATION IN THE SYNTHESIS OF ACETIC AND BUTYRIC ACIDS BY BUTYRIBACTERIUM RETTGERI

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Butyribacterium rettgeri causes a modified butyric acid type fermentation of lactate, the main products being carbon dioxide and acetic and butyric acids.[1] This fermentation is remarkable because of the low yield of carbon dioxide and the high yield of fatty acids. In the usual type of butyric acid fermentation, such as is carried out by *Clostridium saccharobutyricum*,[2] for example, not less than one mole of carbon dioxide and not more than one mole of C_2 derivatives (acetic acid, butyric acid $\times$ 2, etc.) are formed per mole of triose. *B. rettgeri*, however, produces only 0.4 mole of carbon dioxide and up to 1.2 moles of C_2 derivatives per mole of lactate. These unusual yields suggest that this organism utilizes part of the carbon dioxide produced from lactate for the synthesis of fatty acids. This possibility has been investigated using the long-lived radioactive carbon isotope, C^{14}, as a tracer to follow the transformations of carbon dioxide, with the results reported below.

Experimental.—To investigate the possible conversion of carbon dioxide into fatty acids, *B. rettgeri* was allowed to grow in a medium containing lactate and C^*O_2. (C^* indicates carbon labeled with C^{14}.) The substrate used and the products formed were estimated quantitatively and the C^{14} content of each compound was determined.

A basal medium of the following composition in g. per 100 ml. was used: sodium lactate 0.5–2.0, Difco yeast extract 0.3, yeast autolysate 0.3,

$(NH_4)_2SO_4$ 0.05, K_2HPO_4 0.1, KH_2PO_4 0.4, $MgSO_4.7H_2O$ 0.02, cysteine hydrochloride 0.05, $FeSO_4.7H_2O$ 0.0005, pH 6.2. A sterile solution of labeled sodium carbonate was added to the basal medium after autoclaving to give a final pH of 7.0–7.6. After inoculating with *B. rettgeri*, strain 32, samples were removed to determine the initial lactate and carbon dioxide. An Oxsorbent seal was then applied to remove oxygen. Cultures were incubated at 30–35°C. until growth ceased and the products of the fermentation were estimated quantitatively, separated and their C^{14} contents determined. The separation of the fatty acids was accomplished by the distillation procedure of Schicktanz, *et al.*[3] The method of determining C^{14} activity has already been described.[4]

TABLE 1

THE FERMENTATION OF LACTATE IN THE PRESENCE OF C^*O_2

(Experiment 1)

COMPOUND	MM/10 ML.	CTS./MIN./MM	TOTAL CTS./MIN.
Lactic acid, decomposed	0.463	...	...
Carbon dioxide, initial	0.115	33,800	3,890
Carbon dioxide, final	0.298	4,800	1,430
Acetic acid	0.252	2,470	620
Butyric acid	0.177	6,350	1,120

The results of a typical experiment are given in table 1. It can be seen that approximately 63% of the C^{14} was lost from the carbon dioxide during the fermentation of 0.46 mM of lactate per 10 ml. Of the total C^{14} lost, about 25% and 46% was recovered as acetic and butyric acids, respectively. In another experiment the corresponding values were 26% and 53%, while, in addition, 1% of the C^{14} was recovered as caproic acid, 7% as trichloracetic acid insoluble cell material and 3% as unidentified nonvolatile compounds. The total recovery of transformed C^{14} varied from 80 to 90%.

The distribution of C^{14} in the acetic acid was determined by the decarboxylation of the barium salt as previously described[4] after the purity of the product was established by a Duclaux distillation. The data given in table 2 show that both methyl and carboxyl groups contain the isotope,

TABLE 2

DISTRIBUTION OF C^{14} IN ACETIC ACID

(The figures give the percentage of the total C^{14} in each atom)

CARBON ATOM	EXPT. 2	EXPT. 3
Carboxyl	56.5 ± 2	57 ± 1
Methyl	43.5 ± 2	43 ± 1

though the amount is not the same in the two positions. The carboxyl group contains significantly more C^{14} than the methyl group.

The approximate distribution of C^{14} in butyric acid was determined by a combination of two methods: (1) the oxidation of the ammonium salt with hydrogen peroxide as described by Wood, *et al.*,[5] and (2) the decarboxylation of barium butyrate.

Wood, *et al.*, have shown that the acetone formed in the H_2O_2 oxidation of butyric acid is derived from the alpha, beta and gamma carbon atoms. By further oxidizing the acetone with alkaline iodine solution, iodoform and acetic acid are formed. The iodoform originates from the alpha and gamma positions in butyric acid and may be used to determine the average activity of these two carbon atoms. The acetic acid originates from the alpha or gamma and beta positions. When the molar activities of the iodoform and acetic acid are known, the activity of the carboxyl carbon in acetic acid, which is equivalent to the beta position in butyric acid, can be calculated by difference.

Decarboxylation of barium butyrate was used to determine the activity of the carboxyl carbon. The reaction was carried out at about 400°C. Control experiments with carboxyl-labeled butyric acid, prepared by Gri-

TABLE 3

DISTRIBUTION OF C^{14} IN BUTYRIC ACID

(Experiment 3)

CARBON ATOM	PERCENTAGE OF TOTAL C^{14}
Carboxyl	39.3
Alpha and gamma (average)	30.8
Beta	29.9

gnard synthesis,[6] showed that approximately half (actually 52%) of the carboxyl activity appeared in the barium carbonate formed by decarboxylation. The reaction is therefore reliable for quantitatively determining the amount of C^{14} in the carboxyl group of the fermentation butyric acid.

The results presented in table 3 demonstrate that all four positions in butyric acid contain carbon derived from carbon dioxide. As in acetic acid, the distribution of C^{14} in butyric acid is not entirely uniform. The carboxyl carbon atom contains significantly more C^{14} than the atoms in other positions. The appearance of more C^{14} in carboxyl carbon relative to alpha and gamma carbon was to be expected, since butyric acid is probably formed (see below) by a condensation of two molecules of acetic acid or derivatives thereof,[5, 7] and the methyl group of the acetic acid from which the alpha and gamma carbon atoms would be derived, contains less C^{14} than the carboxyl group. On the same basis, the beta carbon atom of butyric acid should have the same C^{14} content as the carboxyl group, which is not in accordance with the observed result. A possible explanation for this apparent discrepancy is that the reported C^{14} content of the beta carbon atom may be in error. Due to the fact that this value is calcu-

lated by difference from the data for acetic acid and iodoform derived from the oxidation of acetone, it is probably less reliable than the values for the other carbon atoms.

Having demonstrated the conversion of carbon dioxide to fatty acids and cell material, it seemed desirable to determine the total quantity of carbon dioxide formed per mole of lactate decomposed. It is obvious that the total carbon dioxide production ($CO_{2\ total}$) must exceed the observed or net carbon dioxide production ($CO_{2\ obs.}$) by the amount used for synthetic reactions ($CO_{2\ used}$), i.e.,

$$CO_{2\ total} = CO_{2\ obs.} + CO_{2\ used.} \qquad (1)$$

The carbon dioxide used and therefore the total carbon dioxide produced can be calculated from data, such as is presented in table 1, showing the decrease in C^{14} content of the carbon dioxide during the fermentation of a known amount of lactate, if it is assumed that the formation of carbon dioxide from lactate is the only process causing a dilution of the C^*O_2. More specifically, it must be assumed that no exchange of C^{14} occurs between carbon dioxide and any of its reduction products such as acetic and butyric acids.

The absence of such exchange was verified by an experiment in which lactate was fermented in the presence of acetic acid labeled in both the methyl and carboxyl groups; the carbon dioxide was unlabeled. After the fermentation, no C^{14} (2 ± 3 cts./min.) could be detected in carbon dioxide, while about 57% of the C^{14} was present in butyric acid (445 ± 10 cts./min.). Incidentally, this result also proves that butyric acid is formed from acetic acid.

Another assumption involved in the calculation is that the carbon dioxide inside and outside the cells is in isotopic equilibrium at all times. No direct test of the validity of this assumption has been attempted. If isotopic equilibrium does not exist, the C^{14} content of carbon dioxide inside the actively metabolizing cells will be lower than that outside. Such a condition would result in a lowering of the calculated total carbon dioxide production since the utilization of C^*O_2 and hence its dilution would be less than anticipated.

The calculation of $CO_{2\ used}$ and $CO_{2\ total}$ follows the method developed for the acetic acid fermentation caused by *Clostridium thermoaceticum*[4] with the exception that the quantity V representing the carbon dioxide concentration throughout the fermentation is variable instead of constant. At any instant, more carbon dioxide is being produced from the decomposition of lactate than is being used in synthesis; consequently, a net increase in carbon dioxide concentration is observed. Let x be the quantity of C^*O_2 per unit volume at any time during the fermentation, x_i and x_f denoting the initial and final C^*O_2 concentrations. V will now represent the

total concentration of carbon dioxide ($C^*O_2 + CO_2$) present at any time. For every mole of carbon dioxide observed to be produced ($CO_{2\ obs.}$) as a result of the breakdown of lactate, A moles of $CO_2 + C^*O_2$ are converted to acetic acid. The decrease in C^*O_2 concentration ($-\Delta x$) is given by the expression

$$-\Delta x = (A\,\Delta V/V + \Delta V)\cdot x. \qquad (2)$$

The differential form of this relation

$$-dx/x = A\,dV/V \qquad (2a)$$

is integrated between the limits x_i and x_f for x, and V_i and V_f for V, where V_i and V_f denote the initial and final observed concentrations of carbon dioxide.

Thus

$$-\int_{x_i}^{x_f} dx/x = \ln x_i/x_i = A\int_{V_i}^{V_f} dV/V = A \ln V_f/V_i \qquad (3)$$

or

$$A = \log x_i/x_f/\log V_f/V_i. \qquad (3a)$$

Using the data of table 1 where $V_i = 0.115$ mM, $V_f = 0.298$ mM, $x_i = 3{,}890$ cts./min., $x_f = 1{,}430$ cts./min., and the lactate decomposed is 0.463 mM,

$$A = \log 2.72/\log 2.59 = 0.435/0.413 = 1.05$$

and $CO_{2\ total} = 0.395$ mM $+ 0.395 \cdot 1.05$ mM $= 0.81$ mM/mM lactate. In three other experiments the values of A ranged from 0.91 to 1.02 and the values of $CO_{2\ total}$ from 0.62 to 0.81. The lower values were obtained from experiments in which higher concentrations of lactate were fermented (1.0–1.3 mM/10 ml.). However, the data are too few to permit generalization concerning the relation between the quantity of lactate fermented and the functions in question.

Although the above results demonstrate that much more carbon dioxide is produced than accumulates, nevertheless the calculated total carbon dioxide production appears to be significantly lower than one mole per mole of lactate that would be expected on theoretical grounds. This result may well be due to the fact that the carbon dioxide inside the fermenting cells is not in isotopic equilibrium with the carbon dioxide in the outside medium. It has already been noted that lack of isotopic equilibrium would lower the calculated carbon dioxide utilization and total production below the true values.

From the data obtained in these experiments, the fermentation of lactate by *B. rettgeri* can be fitted into the following simplified reaction scheme if we disregard the minor quantitative discrepancies noted above.

$$CH_3CHOHCOOH + H_2O = CH_3COOH + CO_2 + 4H \quad (4)$$

$$2CO_2 + 8H = CH_3COOH + 2H_2O \quad (5)$$

$$2CH_3COOH + 4H = CH_3CH_2CH_2COOH + 2H_2O \quad (6)$$

Reaction (4) represents the oxidation of lactic acid to approximately one mole each of carbon dioxide and acetic acid; the primary product may well be acetyl phosphate or some other C_2 compound convertible into acetic acid rather than the acid itself. Reaction (5) represents the condensation and reduction of two moles of carbon dioxide to acetic acid; this reaction occurs in such a way that both the methyl and carboxyl groups are derived from carbon dioxide. Reaction (6) represents the condensation and reduction of two moles of acetic acid or some related compound to butyric acid. Neither reaction (5) nor (6) goes to completion since they compete for the available hydrogen from reaction (4). The caproic acid which is produced in small yield may be formed by a further condensation of the same type as reaction (6), possibly involving butyric acid and a C_2 compound.[7]

In conclusion it may be pointed out that *B. rettgeri* is the first non-sporulating bacterium and the fourth anaerobe[4, 8, 9] that has been shown to cause a total synthesis of acetic acid from carbon dioxide.

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POLLEN-TUBE GROWTH IN INTERGENERIC POLLINATIONS ON DATURA STRAMONIUM

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During the past 30 or 40 years, research in pollen-tube growth has been carried out by numerous investigators. Pollen has been successfully germinated on artificial media and in a few cases on stigmas of unrelated plants (Yasuda[6] and Eigsti[5]). The rate of pollen-tube growth has been studied especially in relation to self-sterility. In many cases the pollen tubes grew slower in self-pollinations of self-incompatible varieties, so that pollen tubes did not reach the ovary before the styles decayed. In other cases pollen-tube development was found to be the same in both self and cross pollinations. The rate of pollen-tube growth in incompatible interspecific crosses has frequently been shown to be slower than in the compatible crosses. In some of the latter cases, an acceleration in the speed of the tubes was observed as they approached the ovary.

Studies of pollen-tube behavior following intergeneric pollinations have been carried out by several investigators. Yasuda[6] reports variation in the rate of pollen-tube growth in such crosses. He found faster tube growth in pollinations of *Petunia violacea* on pistils of *Solanum Gilo* than on those of *Solanum Melongena*, and also in pollinations of *Callistegia japonica* on *Cucurbita maxima* than on *Helianthus annuus*. Eigsti[5] reports normal growth of tubes of *Reseda* in styles of *Datura stramonium*.

The present research deals with pollen-tube behavior following intergeneric pollinations of *Datura stramonium*. Flowers of this species, Line 1, were used as females. The line is presumably homozygous, having originated from a duplication of the chromosomes of a haploid plant. In the following experiments, ten flowers were pollinated with pollen of each of the plants tested. All the experiments were made at a constant temperature of 26°C. Precautions in pollinations were observed, and preparation of microscopic slides by dissection, staining and mounting of stylar tissue of the pistils was made according to the techniques used by Buchholz.[1] By examination of these slides the extent of growth and behavior of the pollen tubes were determined. The number of normal pollen tubes and of abnormal ones (bursting or swollen) was observed and counted. An index for the speed of the normal-growing pollen tubes was calculated according to the methods used by Buchholz and co-workers.[2] On the basis of pollen germination and of the percentage of normal-growing pollen tubes, the species belonging to the Solanaceae and to other families were separated into the following classes showing: (*a*) no pollen germination; (*b*) most tubes

TABLE 1

CLASSIFICATION OF THE TESTED PLANTS ACCORDING TO THE GERMINATION AND THE PERCENTAGE OF NORMAL-GROWING POLLEN TUBES

FAMILIES	(a) NO GERMINATION	(b) MOST TUBES BURST	(c) ± HALF TUBES GROW	(d) MOST TUBES GROW
Solanaceae	*Nierembergia rivularis*	*Lycopersicum esculentum*	*Browallia speciosa*	*Brunfelsia calycina*
			Brunfelsia nitida	*Capsicum grossum*
			Lycium halimifolium	*Datura stramonium*
			Nicotiana glutinosa	*Nicotiana glauca*
			Physalis ixocarpa	*Nicotiana Langsdorffii*
			Petunia axillaris	*Nicotiana Tabacum*
			Solanum Melongena	*Salpiglossis sinuata*
Acanthaceae	*Ruellia Devosiana*			
Balsaminaceae	*Impatiens sultani*			
Campanulaceae	.....	.....	.....	*Campanula carpatica*
Commelinaceae	*Tradescantia fuscata*			
	Tradescantia virginiana			
Compositae	*Catananche caerulea*			
	Rudbeckia hirta			
Convolvulaceae	*Convolvulus mauritanicus*			
Crassulaceae	*Sempervivum Moggridgei*			
Cucurbitaceae	*Cucurbita maxima*			
Gentianaceae	.....	.....	.....	*Exacum affine*
Geraniaceae	*Geranium sessiliflorum*			
Gesneriaceae	*Achimenes longiflora*			
	Saintpaulia ionantha			
	Sinningia speciosa			
	Streptocarpus kewensis			

Hippocastanaceae	*Aesculus parviflora*			
Labiatae	*Salvia fulgens*	.....	.....	*Stachys discolor*
Leguminosae	*Swainsona galegifolia*			
Linaceae	*Linum alpinum*			
Lythraceae	*Lythrum salicaria*			
Malpighiaceae	*Thryallis glauca*			
Malvaceae	*Althaea rosea*	.....	*Abutilon megapotamicum*	
	Hibiscus Rosa-sinensis			
Onagraceae	*Fuchsia speciosa*			
	Oenothera fruticosa			
Plumbaginaceae	*Plumbago Larpentae*			
Polemoniaceae	*Phlox paniculata*			
Portulacaceae	*Portulaca grandiflora*			
Primulacae	.....	*Anagallis linifolia*		
Ranunculaceae	.....	.....	.....	*Cimicifuga cordifolia*
				Ranunculus anemonoides
Rosaceae	*Potentilla fruticosa*			
	Geum grandiflorum			
Saxifragaceae	*Heuchera sanguinea*			
Scrophulariaceae	.....	.....	.....	*Digitalis ambigua*
				Pentstemon Digitalis
Ternstroemiaceae	.....	*Camellia japonica*		
Amaryllidaceae	*Alstroemeria aurantiaca*	.....	*Narcissus Pseudo-Narcissus*	
Gramineae	*Zea mays*			
Iridaceae	.....	*Crocus biflorus*	*Gladiolus Lemoinei*	*Freesia wisteria*
Liliaceae	*Lilium tigrinum*	.....	*Hyacinthus myosotis*	*Tulipa Gesneriana*

bursting; (*c*) about an equal number of tubes growing and bursting, and (*d*) most tubes growing. The classes (*b*), (*c*) and (*d*) above are characterized by the following percentages of normal-growing pollen tubes: less than 45%, from 45 to 65%, and more than 65%, respectively.

Eighteen-Hour Test of Pollen-Tube Growth.—Styles of *Datura stramonium* were pollinated with pollen of 16 species of Solanaceae and 49 species of 31 other families. The growth and percentages of normal and bursting tubes were studied after 18 hours.

A classification of the tested plants into the four previously mentioned groups according to the pollen germination and to the percentages of normal-growing pollen tubes on pistils of *Datura stramonium* is given in table 1. It can be seen that the pollen of members of the Solanaceae more closely approached normal behavior. Most interest, however, should be attached to those cases of unrelated pollens which fall into the two top classes having 50% or more of normal-growing pollen tubes.

Tests for More Extended Periods of Time.—To determine whether or not normal pollen tubes observed in the 18-hour test would reach the ovary if given more time, some experiments were carried out in which the pollen tubes were allowed to grow for 66 hours before examination. The results of these tests are included in table 2. It can be seen that in two cases the earlier speed was approximately maintained. In most cases, however, the speed of growth declined considerably. With some of the species, tests were carried out for longer intervals of time until the styles began to rot at the base (*Petunia axillaris*, *Browallia speciosa*, *Freesia wisteria*, *Salpiglossis sinuata*). In all cases the pollen tubes were found to have stopped or to have practically stopped at some distance from the ovary.

Attempts to Induce the Pollen Tubes to Reach the Ovary.—Tetraploid versus diploid styles: In an attempt to discover a procedure to speed up the pollen-tube growth, tests were made with pollen of five plants on styles of a tetraploid *Datura stramonium*, Line 1, and the results were compared with those of the diploid styles of the same species, as shown in table 3. Most of the tubes burst when pollen of tomato (*Lycopersicum esculentum*) was used on diploid styles, but most of them grew when tetraploid styles were used. In all but one of the other cases some alleviation of the bursting was noted but to a much less degree. In all five cases growth was slightly increased in the tetraploid styles. However, the most striking effect was seen in the prevention of bursting in tomato.

Style splicing and style mutilation: Some methods to shorten the distance that the pollen tubes have to cover en route to the ovary were tried. The style splicing technique, described by Buchholz and co-workers,[3, 4] was used with some modifications. More promising results, however, were obtained with style mutilations. Pollinations were made inside vertical slits cut in the tops of the decapitated styles. When this technique was used

pollen tubes were induced to grow nearer to the ovary than in uncut styles. Pollen tubes of *Freesia wisteria*, of the Monocotyledoneae, were able to

TABLE 2

PERCENTAGE AND GROWTH OF NORMAL POLLEN TUBES OBSERVED AFTER 18 HOURS, AND OBSERVED AND CALCULATED AFTER 66 HOURS

FAMILIES	SPECIES	PERCENTAGE OF NORMAL TUBES	GROWTH IN 18 HOURS	GROWTH IN 66 HOURS	CALCULATED GROWTH IN 66 HOURS
Solanaceae	*Browallia speciosa*	59.4	7.1	8.0	25.5
	Brunfelsia calycina	88.8	13.7	18.8	49.3
	Brunfelsia nitida	46.3	19.7		
	Capsicum grossum	79.7	2.7		
	Datura stramonium	86.1	42.0		
	Lycium halimifolium	63.6	14.6		
	Lycopersicum esculentum	27.1	13.7		
	Nicotiana glauca	94.6	4.5	16.0	16.2
	Nicotiana glutinosa	63.1	9.0		
	Nicotiana Langsdorffii	90.9	10.7		
	Nicotiana Tabacum	87.1	17.7		
	Petunia axillaris	52.2	6.8	10.0	24.4
	Physalis ixocarpa	47.4	3.0	5.0	10.8
	Salpiglossis sinuata	88.2	18.6	20.0	66.9
	Solanum Melongena	58.3	7.1	12.0	25.5
Other Dicotyledoneae:					
Campanulaceae	*Campanula carpatica*	74.6	10.5		
Cucurbitaceae	*Cucurbita maxima*	6.8	1.8		
Gentianaceae	*Exacum affine*	70.9	5.5		
Labiateae	*Stachys discolor*	74.1	6.3		
Malvaceae	*Abutilon megapotamicum*	59.8	6.8		
Primulaceae	*Anagallis linifolia*	29.7	7.6		
Ranunculaceae	*Cimicifuga cordifolia*	90.0	2.4	27.7	27.8
	Ranunculus anemonoides	83.8	1.5		
Scrophulariaceae	*Digitalis ambigua*	84.6	4.2		
	Pentstemon Digitalis	90.7	12.3		
Terntroemiaceae	*Camellia japonica*	32.0	3.5		
Monocotyledoneae:					
Amaryllidaceae	*Narcissus Pseudo-Narcissus*	46.2	0.6		
Iridaceae	*Crocus biflorus*	25.0	0.6		
	Freesia wisteria	69.6	6.0	15.0	25.2
	Gladiolus Lemoinei	52.3	6.8		
Liliaceae	*Hyacinthus myosotis*	54.5	0.6		
	Tulipa Gesneriana	71.5	7.1		

reach the ovary, although they did not enter it. Pollen tubes of *Petunia axillaris*, *Salpiglossis sinuata* and *Browallia speciosa* grew closer to the ovary than on uncut styles examined when they began to rot at the base.

Summary.—In attempts to obtain intergeneric crosses, many barriers have been found which make these crosses unsuccessful. In this paper some of the barriers connected with pollen germination and with pollen-tube growth have been studied. All tests were made on stigmas and styles of *Datura stramonium*.

The pollens of members of the Solanaceae germinated more successfully than those of other families (93% against 43%). The fact that the pollen of species belonging to such distant groups as the Monocotyledoneae germinated on stigmas of *Datura stramonium* appears to indicate that pollen germination is not determined exclusively by taxonomic relationships.

On the average, the Solanaceae showed faster pollen-tube growth than members of other families (10.8 mm. against 5 mm.). However, in the latter group individual cases of relatively rapid growth of pollen tubes were found.

TABLE 3

FAMILIES AND SPECIES	PERCENTAGE OF NORMAL TUBES 2n	GROWTH 2n	PERCENTAGE OF NORMAL TUBES 4n	GROWTH 4n
Solanaceae				
Lycopersicum esculentum	**27.1**	**13.7**	**80.8**	**15.3**
Petunia axillaris	52.2	6.8	55.6	10.3
Physalis ixocarpa	47.4	3.0	75.6	3.8
Salpiglossis sinuata	88.2	18.6	84.7	25.6
Terntroemiaceae				
Camellia japonica	32.0	3.5	37.4	5.7

The use of tetraploid styles increased slightly the speed of the pollen tubes and decreased the percentage of bursting pollen tubes, especially in tomato, thereby increasing the chances for the tubes to reach the ovary.

Attempts to shorten the distance from the stigma to the ovary induced the pollen tubes to approach closer to the ovary. In one case the tubes arrived at the ovary, but in none did they enter it.

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CYCLES IN MEASLES AND CHICKEN POX

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Persons trained in mathematical physics or astronomy are apt to look for periods in oscillating phenomena and the methods of analysis (such as the periodogram) which have been developed by them for the determination of unknown periods are presumably well suited to eliminate efficiently the random fluctuations of phenomena which do really have an underlying period of unchanging length and phase. It is somewhat doubtful whether epidemiological or economic phenomena have any important periodic components except the seasonal or other variations impressed upon them by major astronomical periods, and it is presumably better that the more general term cycle be used for their ups and downs.[1]

In table 1 are given the reported cases of measles and of chicken pox in eleven cities for nineteen epidemic years.[2] For chicken pox the years are not the same for all cities. The epidemic year for these diseases is taken to run from September 1 to August 31. The number of turning points, as defined by Wallis and Moore,[3] is tabulated in the last row indexed *TP*. A downward or upward phase (W&M, p. 4) lies between two successive turning points and two successive phases form a cycle. The number of phases is thus one less than the number of turning points and the number of cycles is one-half of the number of phases. For measles the number of turning points averages 13.2, the number of phases 12.2 and the number of cycles 6.1. For chicken pox the number of turning points averages 12.9, the number of phases 11.9 and the number of cycles 6.0. The results are equivalent in view of the sampling errors.

The number of phases of 1, 2, 3, . . . years duration can be enumerated and compared with the number that should be obtained for eleven series of nineteen items by chance (W&M, p. 10). It will be observed that both diseases have more phases than would occur by chance on the average. The number of phases can be regarded as normally distributed (W&M, p. 32) about 10.3 with variance[4] 3.06 and the sum of eleven series should be distributed about 113.7 with variance 33.61 or standard deviation 5.80, whereas the actual deviation is 20 for measles and 17 for chicken pox.

The result is therefore very significant; the ups and downs of measles and chicken pox in successive epidemic years are more frequent than in a chance series.

TABLE 1.

REPORTED CASES OF MEASLES AND CHICKEN POX IN ELEVEN CITIES FOR NINETEEN EPIDEMIC YEARS, SEPTEMBER 1 TO AUGUST 31, BEGINNING ON SEPTEMBER 1 OF THE YEAR GIVEN

YEAR	CHIC.	CLEV.	DETR.	L. A.	MILW.	MONT.	N. Y.	PHIL.	PITT.	S. F.	TOR.*
					Measles						
1921	10,845	5,838	6,866	165	538	2281	41,478	3,492	3278	303	5,001
1922	15,878	6,516	4,004	3,638	10,941	2264	12,722	26,406	9220	1,954	2,834
1923	5,412	2,392	4,334	5,051	717	1019	35,311	3,655	855	3,853	1,454
1924	13,992	500	515	899	7,468	3783	4,571	7,703	7698	220	7,260
1925	5,324	15,865	16,520	440	4,242	1223	44,973	13,032	2987	2,707	2,191
1926	19,406	206	414	11,064	3,410	2300	1,724	949	2563	3,951	4,032
1927	1,202	2,589	15,321	672	191	4562	34,758	15,609	5973	817	2,360
1928	17,985	11,266	2,712	604	13,835	2138	3,100	1,450	1180	186	7,226
1929	1,131	321	15,153	6,650	437	2445	22,999	4,926	5365	7,558	660
1930	12,126	3,730	787	3,104	4,977	6450	27,072	15,705	2274	1,042	1,688
1931	9,749	15,097	12,836	456	14,497	4724	8,634	339	6204	3,775	10,208
1932	8,875	110	9,319	9,582	143	534	37,953	6,289	170	116	132
1933	9,379	3,693	2,004	1,142	2,500	4009	4,939	19,514	4388	4,426	78
1934	24,636	7,872	27,694	1,540	9,843	9366	27,782	1,319	9917	1,454	16,110
1935	541	2,346	737	9,210	321	3372	37,080	11,609	661	7,637	1,313
1936	3,995	5,582	1,220	677	371	6524	12,218	785	3256	202	3,707
1937	39,696	6,975	31,074	1,110	27,922	1817	34,624	15,740	7829	145	1,013
1938	572	202	741	9,377	158	9461	3,824	1,211	98	10,435	10,382
1939	2,169	163	4,047	604	3,284	1691	6,186	2,769	104	149	190
TP	14	11	15	11	12	12	15	14	14	13	14
					Chicken Pox						
1921	..	..	..	..	..	539	5,313	3916	..	..	..
1922	3915	..	..	..	..	577	8,469	2753	2015	..	..
1923	5713	3103	3130	3127	2936	794	7,959	5662	2806	1316	2098
1924	4054	3536	2400	2095	1902	596	7,729	3314	2345	1085	1777
1925	4794	1861	2276	1983	3907	1170	6,506	4634	1340	1640	2565
1926	4462	4000	3742	2197	3449	604	10,117	4651	2495	1511	2390
1927	4128	2140	1882	2569	2729	787	6,195	3307	1190	2566	2781
1928	4756	3887	3905	2523	4028	1565	9,764	4392	1875	894	2454
1929	4977	5101	3108	2306	5534	2802	7,939	3762	1771	1778	4233
1930	4934	5897	4244	2032	4789	2528	9,734	4993	2608	1658	3256
1931	4104	3623	2440	3950	2898	2147	7,763	3968	1809	2228	1950
1932	6953	7356	4769	2320	7648	3516	12,463	5937	3341	2201	4169
1933	6206	4665	3168	2754	6511	3667	11,847	4535	2600	2252	2620
1934	5727	5550	3977	3205	3364	4438	10,965	6474	2703	3336	4051
1935	6453	4615	4135	2025	9003	3193	8,180	4072	1939	1580	4548
1936	6356	4627	4611	3442	5552	4898	13,112	6771	3101	2994	4462
1937	6382	4394	3651	3194	3560	3303	10,852	6387	2037	1946	2450
1938	6010	4132	3323	2847	6002	3862	9,019	5606	2553	2612	3585
1939	7776	4947	3436	2176	5032	5068	12,669	6465	2394	1957	4629
1940	6090	4328	4141	2996	5834	..	..	..	2888	3516	2572
1941	..	5062	3096	4270	5807	..	..	..	..	2092	4141
TP	13	14	12	9	12	10	12	15	16	16	13

* The cities are Chicago, Cleveland, Detroit, Los Angeles, Milwaukee, Montreal, New York, Philadelphia, Pittsburgh, San Francisco, Toronto.

For a chance series there is a definite average duration of a phase (W&M, p. 10); with N = 19 it is 1.46. If we double this to get the average length

of a cycle we should have 2.92. The variance of the duration of phase is 0.51. The average duration for measles calculated[5] from table 2 is 1.28 and for chicken pox 1.31. This again shows that the oscillations are more frequent than they would be in a chance series. The differences $1.28 - 1.46 = -0.18$ and $1.31 - 1.46 = -0.15$ are both significant statistically. It should be especially noted that a chance series would have a mean duration of phase which would not be very different from the essentially equal values found for the mean duration in measles and in chicken pox; but our series involving eleven cities for nineteen years is long enough to show the significance of the difference between the observed mean and the theoretical mean. If we double the mean length of the phase to obtain a mean length of the cycle we find just over two and a half years for both measles and chicken pox.

It is characteristic of chicken pox and measles that the seasonal variation in reported cases is great, there being very few reported during the summer. The amount of measles varies so greatly between epidemic years that the epidemic nature of the disease is apparent despite the large seasonal fluctuation; but the variation of chicken pox from one epidemic year to another

TABLE 2

FREQUENCY OF PHASE DURATIONS

DURATION	1	2	3	ABOVE 3	TOTAL
Measles	98	35	1	0	134
Chicken Pox	99	24	8	0	131
Chance	73.3	30.2	8.1	2.0	113.7

is small enough so that one might be tempted to infer that the disease was essentially seasonal and otherwise non-epidemic. It, of course, is quite obvious that the intensity of the epidemic waves is far more pronounced in measles than in chicken pox; the reality of the epidemic character of chicken pox is, however, reasonably clear if one judges by the deviation of the mean duration of phase from that due to chance or from the excess of the number of phases compared with chance.

If table 2 for the frequency of phases of different durations for measles and chicken pox be treated as a contingency table and χ^2 be computed, we find $\chi^2 = 7.5$ for two degrees of freedom which would be formally significant —chiefly because of the relatively great differences in the numbers of phases of three years' duration. Thus there seems to be a slight difference between the two diseases in that chicken pox has more three-year phases than measles and possibly fewer two-year phases (the items might well be correlated).

Wallis and Moore (p. 39) state that their method is not very sensitive to a trend in the data; there is no doubt that there is trend in the reported cases from some cities but we have seen no evidence that the trend is suffi-

	OBSERVED			EXPECTED		
Measles	98	35	1	99.6	29.8	4.55
Chicken Pox	99	24	8	97.4	29.2	4.45

ciently marked to make its elimination important in the application of an insensitive test. If one proceeds to moving averages, thus reducing the amplitude of the variations, it is highly probable that the test might be less insensitive to some general underlying trend. We have, however, ventured to set up the tables for two-year and three-year moving averages and to tabulate the frequency of phases of different duration (table 3). It will

TABLE 3

FREQUENCY OF PHASE DURATIONS IN ONE-, TWO- AND THREE-YEAR MOVING AVERAGES

DURATION	1	2	3	ABOVE 3	TOTAL	MEAN
Original Data (Single Years)						
Measles	98	35	1	0	134	1.28
Chicken Pox	99	24	8	0	131	1.31
Chance	73	30	8	2	114	1.46
Two-Year Moving Average						
Measles	52	41	4	1	98	1.53
Chicken Pox	39	32	7	4	82	1.77
Chance	69	28	8	2	106	1.46
Three-Year Moving Average						
Measles	79	15	9	2	105	1.39
Chicken Pox	80	7	8	5	100	1.42
Chance	64	26	7	2	99	1.46

be observed that for both moving averages in the case of measles the number of phases approaches that due to chance more closely than for the original data; the number of phases in the two-year moving average for chicken pox is notably short of the chance mark. The average duration of phase is also close to the chance value in all cases except the two-year moving average for chicken pox. In the moving averages there tends to be an excess of long phases as compared with the chance distribution or with that of the original data—possibly a reflection of trends in the data, though we have been unable to convince ourselves that it is. There seems to be no clear indication that the actual frequency distributions for the two-year or three-year moving averages are closer to the chance distributions than those for the single years.[6] Moreover, if the phenomenon is cyclic, rather than periodic, there is no obvious reason why the use of a moving average closer to the average length of the cycle, when the standard deviation of the length of a cycle is more than one-third of its mean, should be effective in bringing the frequency distribution close to those due to chance.

[1] Wilson, E. B., *Quarterly Jour. Economics*, **48**, 375–417 (1934); *Science*, **80**, 193–199 (1934).

[2] Dr. Mildred Wells, incident to a discussion of seasonal variation, *Am. Jour. Hyg.*, **40**, 279–317 (1944), has collected reported cases of some of the children's diseases by months in a large number of states and cities for twenty consecutive years and kindly sent us the figures. We have merely combined the monthly data into those for epidemic years.

[3] Wallis, W. A., and Moore, G. H., *A Significance Test for Time Series and Other Ordered Observations*, National Bureau of Economic Research, New York, 1941, 59 pages. We shall have to assume that this monograph is available to the reader, for we cannot take the space to reproduce the definitions and results we used; it will be cited as W&M.

[4] The variances actually observed between the eleven cities are 2.16 for measles and 5.09 for chicken pox, their average is 3.62; their ratio is 2.36 which for 10 degrees of freedom in each is not significant.

[5] Table 2 gives the total enumeration for the eleven cities. If the average duration is found for each city, we have as a grand average 1.29 for measles and 1.34 for chicken pox. (The average of the averages is not quite the same as the average for the total data because the number of phases differs.) The standard deviation of the eleven averages about their mean is for measles 0.14 (variance 0.019) and for chicken pox 0.21 (variance 0.044). The chance variance of 0.51 for the duration of phase would give a chance variance of 0.047 for the average duration of phase for 11 cities; this is near the variance found for chicken pox but about twice that found for measles. If we estimate the standard deviation of the means of eleven cities from the observed standard deviations we have 1.29 ± 0.042 and 1.34 ± 0.063. If we use table 2 we find variances of 0.22 for measles and 0.34 for chicken pox which for averages of about 130 elements would indicate 1.28 ± 0.040 and 1.31 ± 0.051. Thus the results of working with the eleven observed means and with the overall distribution are consistent. The differences $1.28 - 1.46 = -0.18 \pm 0.040$ and $1.31 - 1.46 = -0.15 \pm 0.051$ are significant; if we use the values obtained by working with the eleven cities namely, $1.29 - 1.46 = -0.17 \pm 0.042$ and $1.34 - 1.46 = -0.12 \pm 0.063$ the result for chicken pox barely escapes significance.

[6] We offer this as a statistical judgment only. W&M discuss significance tests at length with reference to χ^2. If the values of χ^2 are computed from the four classifications of phase, viz., one, two, three and more than three years of duration, despite the small theoretical number 2 in the chance series, we find for measles 18, 13, 9 for single years, two-year, three-year moving averages and for chicken pox 12, 16, 23; measles seems to be improving, chicken pox worsening.